Instruction Manual

Semi-Dry Blotters

Catalogue Number

SD10 Semi Dry Mini, 10x10cm System

SD20 Semi Dry Maxi, 20 x 20cm System

Contents:-

SAFETY PRECAUTION

WHEN USED CORRECTLY, THESE UNITS POSE NO HEALTH RISK.
HOWEVER, THESE UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THIS INSTRUCTION MANUAL.

ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.

THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.
THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.

THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES:
73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992
EN 61010-1:1993/BS EN 61010-1:1993

PACKING LISTS:

	Main Unit Base and Lid	Connecting Leads, Pk/2	Instruction Manual
SD10			
SD20			

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Please contact your supplier if there are any problems or missing items.

Usage Guidance and restrictions:

- Maximum altitude 2,000m.
- Temperature range between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31^{O}C decreasing linearly to 50% relative humidity at 40^{O}C.
- Not for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664.
POLLUTION DEGREE 2, states that: “Normally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected”.

Care and Maintenance:-

Cleaning the Semi Dry Blotting Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60^0 C can cause damage to the unit and components.**

The unit should be thoroughly rinsed with warm water or distilled water to prevent build up of salts but care should be taken not to damage the plate electrodes and vigorous cleaning is not necessary or advised.

Air drying is preferable before use.

The units should only be cleaned with the following:-

Warm water with a mild concentration of soap or other mild detergent.

Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons

The units should not be left to in detergents for more than 30 minutes.

The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:-

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol Alkalis.

RNase Decontamination

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using. RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Instructions:-

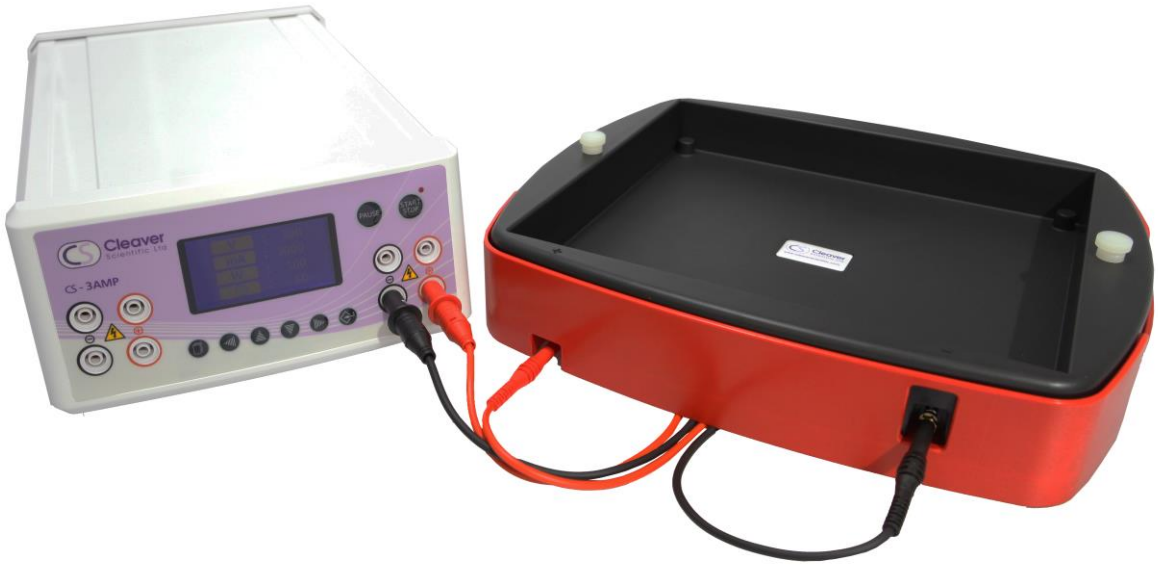
Setting up the blot sandwich:-

Please consult the solutions pages 9 and 10 for details on buffers required for protein, DNA or RNA blotting. The recommended buffers are given on page 9 and alternatives on page 10. This also provides a guide for the amount of standard grade filter paper pieces required for each buffer. Adjust amounts accordingly for thicker grade filter paper.

1. Cut the membrane and filter paper to the size of the gel. **At no point touch the membrane with bare fingers as this will cause changes in the surface properties of the membrane and cause inconsistent sample binding, as well as protein contamination.**
2. Depending on the type of membrane used, the membrane may require equilibration in buffer prior to blotting. This is generally true of PVDF and nylon membranes but varies for nitrocellulose membranes. Please consult the membrane manufacturer's guidelines for wetting the membrane before blotting.
3. Ensure that excess liquid drains from the membrane.
4. Soak the required number of filter paper pieces in the appropriate buffer. Generally six filter paper pieces are required per electrode, twelve in total. Buffers for different types of blotting are listed on pages 9 and 10.
5. Mark or use some other means to enable identification of the gel side of the membrane. This is necessary for the blot probing stage, more efficient binding can occur when the membrane is facing upwards in the probe solution. It is also an idea to clip the corner of the gel nearest to the top of lane 1 was on the gel to allow easy sample identification during analysis.
6. Remove the lid from the blotter and place six pre-soaked filter paper pads onto the base electrode plate ensuring that any excess liquid is wiped away.
7. Carefully place the membrane on top of the six filter paper pads and ensure that no air pockets have formed. Any air pockets should be smoothed out using a wet gloved finger.

8. Place the gel on top of the membrane and smoothen to ensure no air pockets have formed. It may help to add a small amount of transfer buffer to the gel to help the membrane attach to the gel evenly.
9. Place the remaining six filter paper pads on top of the membrane and smoothen gently.
10. Carefully place the lid over the blot sandwich and secure using the screws. These should be tightened evenly a little each at a time. The blot may de disturbed if one screw is tightened fully, then the next. **Note: only use the screws for blotting of acrylamide gels up to 2mm thick. For blotting thicker gels and agarose gels do not use the screws. The weight of the lid will provide enough pressure or a small container of buffer ~ 0.5 – 1 litre container can be used as a weight**
11. Connect the leads to the unit, red to the positive base and black to the negative lid. **Note: The red lead inserts through the lid into the base. The black lead inserts through the side of the base into the lid**. **This is a necessary safety feature so that the electrodes cannot be accessed when the unit is connected to a power supply. (Please see below)**

Figure 1: Recommended Blotter Set Up with CS-3AMP Power Supply



12. Attach the power leads to the appropriate sockets, red to red, black to black on a power supply. **Do not invert the leads or connect up incorrectly as this will cause corrosion of the stainless steel electrode.**
13. Blotting generally requires high current settings >250mA and the power supply should contain these capabilities. Please contact your Cleaver Scientific representative for details of these.

Running the Blot:-

1. The recommended current setting for the Semi-Dry Maxi is between 320 and 800mA; the recommended constant voltage setting from 15V up to 75V. Cleaver Scientific recommends that a time course study is first performed with protein markers to establish the most efficient settings. Higher current settings may cause excessive heat generation and the maximum limits below must never be exceeded:-

SD10 – 550mA, 75 Volts

SD20 - 1200mA, 75 Volts

2. Allow transfer to proceed for between 30 minutes to one hour depending on current settings.
3. The efficiency and quality of transfer depends on the type of buffer used and also the type of samples. Better results are usually obtained by reducing the power settings and increasing the blot time. It is generally best to perform a time course for the type of gel, samples and buffering system being used to optimize transfer efficiency.

References

Western Blotting:-

1. Bjerrum, O.J. and Schafer-Nielsen, C. in: Dunn, J.J. (ed.) **Electrophoresis** '86 VCH Weinheim 1986, pp. 315-327.
2. Tovey, E.R. and B.A. Baldo. 1987. **Comparison of semidry and conventional electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes.** Electrophoresis 8: 384-387.
3. Dunbar, B.S., Ed. 1994. **Protein Blotting**: A Practical Approach. IRL Press at Oxford University Press, Oxford, England.

Southern / Northern Blotting:-

1. "**Blotting, Hybridization & Detection: An S&S Laboratory Manual**", a publication of Schleicher and Schuell.
2. **"Hybond Blotting Guide: The direct route to excellent blotting results**", Amersham Life Science.

General:-

1. Sambrook, Fritsch, and Maniatis, **Molecular Cloning A Laboratory Manual,** Second Edition,
Cold Spring Harbor Laboratory Press, 1989.
2. **Current Protocols in Molecular Biology,** Greene Publishing Associates and Wiley-Interscience,1989.

Solutions:-

NOTE:- Transfer buffers must be made accurately using high grade reagents. Do not adjust the pH with acid or base as this will affect the properties of the buffer.
pH will vary according to the purity of the reagents used.

Protein (Western) Blotting Buffers:-

Triple buffer system:-

For high efficiency transfer of Protein from acrylamide gels

Anode 1 Buffer: 0.3M Tris Base, 20% MeOH, pH 10.4 – Soak 4 standard grade filter paper sheets

Anode 2 Buffer: 0.025M Tris Base, 20% MeOH pH 10.4 – Soak 2 standard grade filter paper sheets

Cathode Buffer: 0.025M Tris Base, 0.04M Caproic Acid, 20% MeOH pH 9.4 – Soak 6 standard grade filter paper sheets

DNA (Southern) Blotting Buffer:-

For high efficiency transfer of DNA from agarose gels.

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:-

50 X 1M ethanolamine-glycine buffer, pH 11

RNA (Northern) Blotting Buffer:-

For high efficiency transfer of RNA from agarose gels.

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:-

50 X 0.2M morpholinopropanesulfonic acid (MOPS)

50mM sodium acetate

5mM EDTA

pH 7.0

General Nucleic Acid Transfer Buffers:-

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:-

1x TAE 40 mM tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA.

50x (1L) dissolve in 750 ml distilled water:

242 g tris base (FW = 121)

57.1 ml glacial acetic acid

100 ml 0.5 M EDTA (pH 8.0).

Fill to 1 litre with distilled water.

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:-

1x TBE 89 mM tris (pH 7.6), 89 mM boric acid, 2 mM EDTA

10x (1L) dissolve in 750 ml distilled water:

108 g tris base (FW = 121)

55 g boric acid (FW = 61.8)

40 ml 0.5 M EDTA (pH 8.0)

Fill to litre with distilled water.

General Transfer Buffers:-

Towbin Buffer with 20% methanol

Soak 12 pieces of standard grade filter paper, 6 for the cathode, 6 for the anode in:-

0.025M Tris Base

0.192M Glycine

20% Methanol.

pH 8.3

TROUBLESHOOTING

Nucleic Acids	
Poor nucleic acid transfer	Transfer apparatus assembled incorrectly and proteins moving in the wrong direction • Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.
DNA / RNA remains within gel	• Gel too hot and buffer too concentrated, resulting in excessive current and the gel starting to melt. Remake buffer to 0.5X TBE, the concentration required for proper transfer • Power conditions changed during transfer. Important to maintain constant current. If buffer less concentrated than 0.5X, higher voltage is required to maintain recommended current, and vice versa. If voltage is too low, current will also fall below optimum setting, reducing migration. Increase voltage limit on power supply. • Optimum transfer of plasmid, vector and PCR DNA achieved using settings recommended within the section: 'Running the Blot'.
Poorly blotted or diffused transfer	• Poor contact between agarose and transfer membrane. Roll out gel with pipette before transfer to remove air and buffer bubbles. Repeat for blotting paper. • Gel too thin, causing uneven electrical contact between the gel stack and electrodes. A 6-mm-thick gel and extra-thick blotting paper are recommended for full electrical contact. • The gel may be too hot. Refer to DNA / RNA remains within the gel. • Very small DNA fragments will diffuse during electrophoresis and blotting even if run in high percentage gels. Resolution is not always improved by running high percentage gels. • Transfer membrane used might not properly bind DNA or RNA. Try a control membrane, a different lot or brand.
Poor Detection Sensitivity	• Poor DNA probe labeling • Insufficient signal hybridized to target DNA for detection. Labeled DNA probe not properly labeled. Check labeling controls to ensure that correct template DNA is being used and that reaction is working properly. • Incomplete transfer of target DNA from gel to membrane. See **'DNA / RNA Remains within the Gel'**. Check agarose gel following transfer to determine whether transfer occurred or not. • Specific activity of the probe may not be high enough for

	standard detection conditions. Determine specific activity and total cpm of probe added during hybridization. • Hybridization conditions may be too stringent; alter to reduce stringency and improve efficiency of probe-template binding.
High Background	• Increase hybridization stringency to reduce non-specific probe binding.
Protein	
Poor protein transfer	Transfer apparatus assembled incorrectly and proteins moving in the wrong direction • Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.
	Western detection system not working or not sensitive enough • Include proper positive or negative control antigen. Consult kit manual. • Use protein markers with coloured reference bands during PAGE. • Stain gel with Coomassie, or stain membrane with Ponceau S.
	Transfer time too short – increase transfer time
	Power setting too low • Check current at beginning of run. Current may be too low for a given voltage setting. Increase current if necessary but do NOT exceed 1200mA. • Buffer may be prepared improperly – prepare new buffer and increase voltage.
	Charge-to-mass ratio incorrect for native transfers. • Proteins close to isoelectric point (pI). Change buffer pH so that it is at least 2 pH unit higher or lower than pI of protein of interest.
	Defective or inappropriate power supply used. • Check fuse of power supply. Ensure max. current output of power supply is at least 2000mA.
	Excessive methanol restricting transfer. • Reduce methanol concentration to maximize protein transfer from gel, but without reducing concentration to the extent that it prevents binding to nitrocellulose. Alternatively reduce methanol concentration and switch to PVDF.
Protein precipitating in gel	• Use SDS in transfer buffer (SDS can increase transfer efficiency, but it can also reduce nitrocellulose binding affinity and affect protein-antibody reactivity). • Remove alcohol from transfer buffer.

Swirls or missing bands; diffuse transfers	Poor gel-membrane contact. Air bubbles or excess buffer remain between membrane and gel. • Carefully remove air bubbles between gel and membrane using a rolling pin • Use more, or thicker, filter paper in gel-membrane sandwich • Replace the fibre pads, as they degrade and remain permanently compressed over time.
	Membrane not fully wet or has dried out • White spots on nitrocellulose membrane indicate dry areas to which proteins will not bind. Ensure membrane is completely immersed in transfer buffer. • If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet. • If using PVDF, immerse membrane in methanol before soaking in transfer buffer.
	Problem with gel electrophoresis. • Poor gel polymerization, inappropriate running conditions, buffer contamination, excessive sample application all contribute to poor quality gels and transfers.
Gel cassette pattern transferred to blot	Contaminated fibre pads • Replace fibre pads or clean thoroughly. Contaminated transfer buffer • Replace buffer solutions.
Poor binding to membrane - nitrocellulose	Excessive methanol restricting transfer. • Ensure methanol concentration does not exceed 20% (v/v).
	Proteins may be transferring through nitrocellulose. • Use PVDF or smaller pore size (0.2µm) nitrocellulose. • Overlay an extra piece of nitrocellulose over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Proteins <15kDa have reduced binding to 0.45µm nitrocellulose or may be washed from membrane during assays. • Use PVDF or nylon membrane, which have higher binding capacities. • Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more stringent washing steps.
	SDS in transfer buffer reducing binding efficiency • Reduce or eliminate SDS concentration
	Membrane incompletely wet • White spots indicate dry areas where protein will not bind.

	• If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet.
Poor binding to membrane PVDF	Membrane is not completely wet • Because of hydrophobicity of PVDF, the membrane must be soaked entirely in methanol before equilibration in aqueous buffer
	Proteins might be transferring through the membrane • Decrease voltage if transferring under high intensity conditions • Overlay an extra piece of PVDF over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Membrane might have dried during handling • Fully wet membranes have a grey translucent appearance. White spots will form on the surface if the membrane has been allowed to dry. As proteins will not bind to dry spots, re-soak the membrane in methanol and re-equilibrate in transfer buffer
	SDS in transfer buffer reducing binding efficiency • Reduce or eliminate SDS concentration
Power is too high	Always check current at the start of the run, for the current might be too high for a given voltage setting. Improper buffer preparation can also result in high conductivity and excessive power generation. The current setting should not be allowed to exceed 2000mA.
Immune-specific detection	Overall high background • Reduce antibody / protein sample concentration Too low background • Increase antibody concentration / protein sample concentration Consult manual included with antibody detection kit
Total protein detection	Consult stain or detection kit manual.

Warranty

Cleaver Scientific Semi-Dry Blotting units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, Cleaver Scientific will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than Cleaver Scientific are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by Cleaver Scientific or its associated distributors have invalidated warranty.

Cleaver Scientific cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

If a problem does occur then please contact your nearest Cleaver Scientific supplier.

Cleaver Scientific Ltd

www.cleaverscientific.com